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Estimation of Oxidative Stress Enzymes and Vitamins Levels in Acute Lymphocytic Leukemia Patients in the Governorate of Basrah, Iraq

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ABSTRACT

Acute lymphocytic leukemia (ALL) is a multi-disease that affects most people, especially children. The present research was conducted to estimate some oxidative stress enzyme and antioxidant vitamin levels in the blood serum of Iraqi ALL patients in relation to sex and age. In this study, 100 patients (57 men and 43 women) with ALL disease and 80 healthy subjects (43 men and 37 women) ranging in age from 1 to 17 years were enrolled. Venous blood was clinically collected from both the ALL patients and healthy individuals. Blood serum levels of glutathione peroxidase (GPx) and superoxide dismutase (SOD) were tested using the enzyme-linked immunosorbent assay. Vitamins A, E, and C were also assessed. The amount of catalase (CAT) in the sample was measured using a spectrophotometric technique. The results revealed that the levels of GPx, SOD, and CAT decreased significantly ($P<0.0001$) in ALL patients compared to the control group. Also, the levels of vitamins A, E, and C decreased significantly ($P<0.0001$) in ALL patients compared to the control group. The findings of this study revealed that GPx, SOD, and CAT enzymes, as well as vitamins A, E, and C are considered key biochemical markers of ALL disease.

Keywords: Acute lymphocytic leukemia, Catalase, Glutathione peroxidase, Superoxide dismutase, Vitamin A, Vitamin E

Introduction

Most humans are currently suffering from a variety of health problems as a result of diseases caused by biochemical and physiological abnormalities. Acute lymphocytic leukemia (ALL) is a serious disease that affects people of all ages and is classified as leukemia. The real causes of this disease are a malignant disorder of the bone marrow, the proliferation of early lymphoid precursors, and the replacement of normal hematopoietic living cells in the bone marrow.[1–3] It is well recognized that different therapies are required for cancer treatment. These therapies undoubtedly have a variety of roles in influencing the biochemical system and its biological processes, resulting in multiple gene modifications. Furthermore, these disorders in the human body cause genetic mutations, abnormal blood proteins, abnormal enzyme function, changes in trace element levels, an abnormal antioxidant vitamins, and a change in lipid profile levels that result from this biochemical condition. Therefore, ALL has a variety of complexes in the function of living cells, especially in the biochemical action of chemical molecules found within the cells.

Several studies have been conducted in different countries on the levels of enzymatic antioxidants such as Glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) in individuals affected with ALL disease, in relation to various parameters such as age, sex, family history, and blood group.[4–6] ALL disease has an important biochemical correlation to liver function. Therefore, any disorder resulting from a hepatic injury will produce biochemical and clinical patterns represented by hepatocellular features. This results in a change in the initial levels of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), and alkaline phosphatase (ALP) enzymes. Also, depending on the severity of the disease, the levels of bilirubin and albumin are adjusted. The dangers of ALL necessitate the presence of pharmacological therapies with medical effects to reduce disease inflammation.[7,8]

This study was aimed at estimating the levels of some oxidative stress enzymes and antioxidant vitamin levels in the blood serum of Iraqi ALL patients in relation to sex and age factors.

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Materials and Methods

Experimental grouping

The total number of patients recruited in this study was 100 (57 males and 43 females) while the number of healthy participants (control group) was 80 (43 males and 37 females) with ages ranging from 1 to 17 years. The patients who were suffering from ALL in the Basrah Teaching Special Hospital for Children, Basrah Governorate, Iraq were recruited. All the patients and healthy subjects were divided into three groups: the first (1-6 years), the second (7-12 years), and the third (13-17 years). The clinical test was performed on the patient as well as the control groups that were divided by age, sex, family history, and blood group.

Ethical approval

The ethical approval for the study with the number (1444/7/3) on (23/11/2020) was obtained from the University of Basrah - College of Education for Pure Sciences - Department of Chemistry.

Collection and clinical preparation of blood samples

Two milliliters of venous blood were collected from patients with ALL and healthy subjects. The blood samples were made to clot and centrifuged for 10 minutes at 4000 rpm. Thereafter, all sera from blood samples were maintained at 20°C until required for biochemical parameter evaluation. The rest of the blood was placed in special tubes that do not contain heparin. To separate the blood plasma, the blood samples were centrifuged for eight minutes at 3000 rpm. The

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erythrocytes were washed gently with 9% (w/v) sodium chloride, and the solution was lysed with deionized water at a ratio of 1:1 (v/v).[9,10] The levels of GPx and SOD in the blood serum of ALL patients and healthy individuals were measured using the enzyme-linked immunosorbent assay (ELISA) method, whereas the enzyme CAT was estimated using the spectrophotometric analysis.[11,12]

Estimations of vitamin A, E, and C levels in blood samples

The amounts of non–enzymatic antioxidants represented by vitamins A, E, and C in blood serum from all patients and healthy individuals were measured using the ELISA technique.

Statistical analysis

The data were presented as mean ± standard deviation of the mean. Using the Variance Univariate program, the variables were statistically analyzed. The regression coefficient (r) was used to differentiate between all the means relating to the control and patient groups. The significant variance among ALL patients and control groups was determined using the Statistical Program for Social Sciences (SPSS; version 23). The regression coefficient (r) was used to discriminate between all means belonging to the control and patient groups. At $p < 0.05$, the p-value was considered significant.

Results and Discussion

The presence of diverse oxidative enzymes, antioxidant vitamins, blood proteins, triglycerides, phospholipids, lipoproteins, creatinine, creatine, malondialdehyde, and some trace elements in the blood produces numerous complex changes and biochemical disorders in ALL patients.[13] Table 1 illustrates the biochemical activity of enzymatic antioxidants represented by CAT, SOD, and GPx in the blood serum of individuals with ALL, as a function of age. The levels of CAT enzyme recorded in the serum of ALL patients were 0.18 ± 0.16, 0.14 ± 0.03, and 0.14 ± 0.027 K/mL for the first, second, and third age categories, respectively. Meanwhile, SOD enzyme levels were 1233.84 ± 492, 26,1183.09 ± 512.30, and 1104.63 ± 519.50 pg/mL for the same age category, respectively. However, the levels of GPx enzyme in ALL patients were 26.05 ± 9.80, 24.05 ± 7.09, and 25.28 ± 8.87 pmol/mL in the first, second, and third age categories, respectively. In all age categories, there was a significant ($P<0.0001$) reduction in CAT, SOD, and GP_X enzyme levels in ALL patients compared to control subjects.

Vitamins A, C, and E are non-enzymatic antioxidants with clinical and physiological functions in determining the severity of ALL disease. As a result, the amounts of these vitamins in the serum of ALL patients were determined based on the age factor (Table 2). Concerning the effect of sex on ALL, it was discovered that different levels of vitamin A were recorded at 1012.57 ± 212.07, 998.15 ± 181.49, and 957.97 ± 160.15 pg/mL in the first, second, and third age category, respectively. Meanwhile, different levels of vitamin E were recorded at 8.14 ± 2.75, 7.16 ± 0.67, and 7.55 ± 2.20 pg/mL for the same age catdgory, but different levels of vitamin C were estimated at 9.57 ± 7.86, 8.70 ± 7.30, and 7.23 ± 5.57 pg/mL for the same age category.

The biochemical activity of antioxidant enzymes such as CAT, SOD, and GPx in the serum of individuals with ALL disease in relation to sex factor revealed that the levels of CAT enzyme in ALL patients were found to be 0.17 ± 0.16 and 0.14 ± 0.03 K/mL for males and females, respectively as presented in Table 3. In terms of SOD enzyme levels, ALL patients represented by males and females had values of 1274.73 ± 554.05 and 1378.21 ± 444.76 pg/mL, respectively. Table 3 reveals that the levels of GPx enzyme in ALL patients were 24.15 ± 8.54 and 27.16 ± 9.47 pmol/mL for males and females, respectively. As shown in Table 4, the biochemical effect of the sex factor is very important in estimating vitamins A, E, and C levels in the serum of ALL patients. Vitamin A levels in males and females were found to be 1093.04 ± 254.50 and 1094.57 ± 350.11 pg/mL, respectively, with respect to this antioxidant vitamin. Also, the levels of vitamin E in patients with ALL were found to be 8.08 ± 2.68 and 9.03 ± 3.68 pg/mL for males and females, respectively. Vitamin C levels were observed to be 9.52 7.74 pg/mL in males and 10.98 7.98 pg/mL in females.

Table 1: Levels of antioxidant enzymes in patients with acute lymphocytic leukemia in relation to age

Age category (Year)	Groups	CAT (K/mL)	SOD (pg/mL)	GP_X (pmol/mL)
First	Control (No. = 35)	1.338 ± 0.04	2303.96 ± 222.24	57.15 ± 3.66
(1-6)	ALL patients (No.= 57)	$0.18 \pm 0.16^{***}$	$1233.84 \pm 492.26^{***}$	$26.05 \pm 9.80^{***}$
Second	Control (No. = 25)	1.35 ± 0.05	2287.35 ± 237.30	57.74 ± 4.25
(7-12)	ALL patients (No.= 22)	$0.14 \pm 0.03^{***}$	$1183.09 \pm 512.30^{***}$	$24.05 \pm 7.09^{***}$
Third	Control (No. = 20)	1.344 ± 0.029	2363.05 ± 206.94	58.26 ± 4.56
(13-17)	ALL patients (No.= 21)	$0.14 \pm 0.027^{***}$	$1104.63 \pm 519.50^{***}$	$25.28 \pm 8.87^{***}$

Values were presented as mean±SD; *: $P < 0.05$; **: $P < 0.001$; ***: $P < 0.0001$; CAT: Catalase; SOD: Superoxide dismutase; GPx: Glutathione peroxidase

Table 2: Levels of antioxidant vitamins in patients with acute lymphocytic leukemia in relation to age

Age Category (Year)	Groups	Vitamin A (pg/mL)	Vitamin E (pg/mL)	Vitamin C (ng/mL)
First	Control (No. = 35)	1893.28 ± 227.51	22.00 ± 3.38	34.96 ± 2.04
(1-6)	ALL patients (No.= 57)	$1012.57 \pm 212.07^{***}$	$8.14 \pm 2.75^{***}$	$9.57 \pm 7.86^{***}$
Second	Control (No. = 25)	1903.94 ± 213.81	23.24 ± 3.50	35.61 ± 2.45
(7-12)	ALL patients (No.= 22)	$998.15 \pm 181.49^{***}$	$7.16 \pm 0.67^{***}$	$8.70 \pm 7.30^{***}$
Third	Control (No. = 20)	1943.05 ± 194.93	24.00 ± 3.61	35.62 ± 2.84
(13-17)	ALL patients (No.= 21)	$957.97 \pm 160.15^{***}$	$7.55 \pm 2.20^{***}$	$7.23 \pm 5.57^{***}$

Values were presented as mean ± SD; *: $P < 0.05$; **: $P < 0.001$; ***: $P < 0.0001$

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ALL disease's risk and complications play a variety of biochemical and medicinal roles in human health. One such danger and complication is a rise or reduction in the levels of enzymatic and non–enzymatic antioxidants such as vitamins A, E, and C. CAT, SOD, and GPx are some of the enzymes found in ALL patients serum that serve as antioxidants. As a result, the severity of ALL disease is determined by these biochemical properties.[14] Clinical characteristics such as age and sex have biochemical relationships with biochemical markers such as enzymatic antioxidants and vitamins.[15] In the first, second, and third age category, there was a significant difference ($P < 0.0001$) in CAT enzyme levels between ALL patients and the control group. Also, SOD enzyme levels showed a low significant difference ($P< 0.0001$) between ALL patients and healthy subjects in the same age group, whereas GPx enzyme levels showed a significant decrease ($P < 0.0001$) in ALL patients when compared to the control group in the same age group. Similar discoveries of enzymatic antioxidant depletion levels that have been found in early onset of other types of cancer explain these situations, thus, demonstrating the medical, physiological, and biochemical roles of antioxidant enzymes in cancer disease prevention.[16,17] In the first, second, and third categories of age, there was significant difference ($P < 0.0001$) in vitamin A levels as antioxidants between ALL patients and control group (Table 2). In addition, when comparing ALL patients to healthy individuals (control group) for the same age category, vitamin E levels in ALL patients showed a highly significant decrease ($P< 0.0001$), whereas vitamin C levels in ALL patients showed a high significant difference ($P< 0.0001$) in all age groups. The CAT enzyme levels exhibited a highly significant decrease ($P<0.0001$) in ALL patients compared to healthy subjects (control group) in relation to sex. The maximum levels of CAT were recorded in males, while the minimum was detected in females. The SOD enzyme levels was found to have decreased significantly ($P<0.0001$) in ALL patients when compared to the control group in both males and females. Meanwhile, the highest levels of SOD were measured in females and the lowest in males.

Low levels of GPx and CAT can contribute to the accumulation of hydrogen peroxide in living cells affected by cancer. Also, cancer cells in the human body develop the ability to produce large volumes of hydrogen peroxide. Because this compound is reactive, it reacts with the plasma membrane and other biological molecules, resulting in increased lipid peroxidation.[18,19] Currently, similar findings have been made about DNA base damage and its association with other enzyme antioxidants such as CAT, SOD, and GPx in human serum.[20,21] When males and females affected by ALL were compared to the control group, there was a significant difference ($P<0.0001$) in SOD enzyme activity. Enzymatic antioxidants, such as SOD, have a biochemical role in protecting and defending the biological system against reactive oxygen species. SOD can also dissolve superoxide to form hydrogen peroxide.[22] The sex factor is a very important variable in determining antioxidant vitamin levels. In males and females, vitamin A, E, and C levels were found to be significantly lower ($P<0.0001$) in ALL patients compared to healthy individuals. Vitamins A, E, and C, as antioxidants, react with various classes of free radicals through control of the reaction chain, protecting the biological system from attack by free radicals, particularly reactive oxygen species. Thus, an abundance of vitamins A and E in the cell prevents the accumulation of the reactive radicals.[23]

Table 3: Levels of antioxidant enzymes in patients with acute lymphocytic leukemia in relation to sex

Sex	Groups	CAT (K/mL)	SOD (pg/mL)	GPX (pmol/mL)
Male	Control (No.= 43)	1.31 ± 0.05	2357.55 ± 122.52	56.04 ± 3.84
	Patients (No = 57)	0.17 ± 0.16***	1274.73 ± 554.05***	24.15 ± 8.54***
Female	Control (No.= 37)	1.36 ± 0.01	2494.82 ± 105.61	59.61 ± 4.83
	Patients (No = 43)	0.14 ± 0.03***	1378.21 ± 444.76***	27.16 ± 9.47***

Values were presented as mean±SD; *: $P < 0.05$; **: $P < 0.001$; ***: $P < 0.0001$; CAT: Catalase; SOD: Superoxide dismutase; GPx: Glutathione peroxidase

Table 4: Levels of antioxidant vitamins in patients with acute lymphocytic leukemia in relation to sex

Sex	Group	Vitamin A (pg/mL)	Vitamin E (pg/mL)	Vitamin C (ng/mL)
Male	Control (No.= 43)	1899.92 ± 289.07	22.52 ± 3.15	33.95 ± 2.07
	Patients (No = 57)	1093.04 ± 254.50***	8.08 ± 2.68***	9.52 ± 7.74***
Female	Control (No.= 37)	1998.01 ± 201.57	23.56 ± 3.22	36.93 ± 1.59
	Patients (No = 43)	1094.57 ±350.11***	9.03 ± 3.68***	10.98 ± 7.98***

Values were presented as mean±SD; *: $P < 0.05$; **: $P < 0.001$; ***: $P < 0.0001$

Conclusion

From the findings of this study, the enzymes CAT, SOD, and GPx had modest significant variations in their levels in patients with ALL compared to healthy individuals. In addition, very significant variations in antioxidant vitamins A, E, and C levels in blood serum linked with ALL patients compared to the control group were seen for the age group. The effect of the sex factor was clear when estimating GPx, SOD, and CAT. Significant increases in vitamin A, E, and C were detected when compared to healthy people, with female patients being the most affected when compared to healthy subjects (control group). As a result, the enzymes GPx, SOD, and CAT, as well as vitamins A, E, and C, are regarded as critical biochemical markers of ALL illness.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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